APPENDIX 3.8-4
SOP FOR THE TISSUE REDUCTION/GRINDING FOR WHOLE BODY AND FILLETED FISH (NE132_07)

STANDARD OPERATING PROCEDURE

TISSUE AND PREPARATION & HOMOGENIZATION

FOR BIOTA AND PLANT MATRICES

Reference Methods: US EPA SW-846 Test Methods for Evaluating Solid Waste

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APPROVALS

Dan Pfalzer
Assistant General Manager — Date: 03/29/2011

Christina L. Braidwood
Quality Manager — Date: 03/29/2011

PERIODIC REVIEW

SIGNATURES BELOW INDICATE NO CHANGES HAVE BEEN MADE SINCE PREVIOUS APPROVAL.

Signature	Title	Date
Signature	Title	Date
Signature	Title	Date

PACE ANALYTICAL SERVICES, INC
2190 TECHNOLOGY DRIVE
SCHENECTADY, NY 12308

(518) 346-4592

STANDARD OPERATING PROCEDURE

LABORATORY PROCEDURE NE132_07.DOC

REVISION 7 (03/29/11)

1.0 IDENTIFICATION OF TEST METHOD

1.1 Standard Operating Procedure for tissue preparation, processing and homogenization prior to extraction/digestion and analysis.

2.0 APPLICABLE MATRIX OR MATRICES

2.1 This method is applicable to the preparation and homogenization of animal and plant matrixes; including but not limited to: fish (whole body and fillets), mollusks (mussels, clams, etc.), crustaceans (lobster or shrimp, etc.), mammals (mice, mink, muskrat, shrew etc.), reptiles and amphibians (frogs or turtles, etc.), macro invertebrates (benthic worms, eels, insects and other biota), and vegetation (coastal and wetland grasses/plants).

3.0 DETECTION LIMIT

3.1 Not applicable

4.0 SCOPE AND APPLICATION

4.1 This method is intended to describe the preparation and homogenization procedures prior to the extraction, digestion and/or clean up of sample extracts. This procedure uses a variety of cutting, grinding and scaling equipment for size reduction, composting, and homogenization. Client and/or project may dictate additional specific requirements than stated below. Samples are best processed when partially frozen. Samples may be re-frozen after processing pending extraction or digestion.

5.0 SUMMARY OF TEST METHOD

5.1 Fish

5.1.1 Samples are weighed, measured, and gender determined if possible. The fish may be processed whole body or as fillets, and with the skin on or off. If fillets are to be removed and processed separately, this is generally done after the removal of the skin. If compositing is required, the identified samples for composite are filleted or skinned prior to homogenization. The carcass of the fish (after removal of the fillet) may be maintained for separate homogenization and analysis if requested.

5.2 Mollusks, crustaceans and other like invertebrates

5.2.1 Samples are measured and weighed prior to processing. Mollusks must be removed from their shells before processing. Due to the low weight of a single mollusk, crustacean, or invertebrate, these sample types are generally composited with others of the same species and/or sampling area prior to homogenization. Gender determination may need to be performed, i.e. lobsters. This is done prior to any processing and recorded. Additionally, lobsters are usually dissected, and the edible

meat (tail and claw) is removed for homogenization. Certain internal organs such as the hepatopancreas may need to be processed separately. If crabs are being processed, the legs, claws and body cavity are generally homogenized together.

5.3 Mammals

5.3.1 Mammals such as mink, mice, shrew or other rodents, must be prepared in a glove box or bio-hazard hood with the use of a HEPA biological respirator due to the potential health hazards associated with mammal tissue. All project specific sample preparation (weighing, skinning, compositing and homogenization) is performed in the glove box. Waste from the process must be treated with bleach before disposal. The outside surfaces of the sample containers must be disinfected before removal from the glove box.

5.4 Reptiles and Amphibians

5.4.1 Samples are generally processed as whole body samples. Depending upon the size, the specimen may need to be cut into small pieces and processed in part, then re-combined as a single sample. Due to the thickness of the skin of most reptiles, such as frogs, it is recommended that these be processed without the skin. If the skin must be processed, ensure that the grinder or processor blades are sharpened before use. The blades may need to be re-sharpened between every few samples as needed. Turtles must be removed from the shell prior to processing by digging out the head and legs, and as much of the body as feasible.

5.5 Macro invertebrates

5.5.1 Macro invertebrates such as worms, eels, insects or benthic biota are generally processed as whole body samples. Depending upon the size, the specimen may need to be cut into small pieces and processed in part, then recombined as a single sample. Due to the low weight of a single invertebrate, these sample types are generally composited with others of the same species and/or sampling area prior to homogenization.

5.6 Plants

5.6.1 Samples are rinsed prior to processing to remove soil, silt, small insects or other debris. Depending upon the size of the plant and the leaves, the sample may be processed mechanically, or may have to be cut into small pieces by hand. Plants can be processed either wet or dry, depending upon project specifications

6.0 DEFINITIONS

6.1 Abdomen- the posterior section of the body behind the thorax in an arthropod.

6.2 **Abductor-** to draw or spread away (as a limb or the fingers) from a position near or parallel the median axis of the body or from the axis of a limb.

6.3 **Arthropod-** any of a phylum (Arthropoda) of invertebrate animals (as insects, arachnids, and crustaceans) that have a segmented body and jointed appendages, a usually chitinous exoskeleton molted at intervals, and a dorsal anterior brain connected to a ventral chain of ganglia.

6.4 **Biota-** the flora or fauna of a region.

6.5 **Bivalve-** being or having a shell composed of two valves (shells).

6.6 **Caudal-** directed toward or situated in or near the tail or posterior part of the body.

6.7 **Carapace-** bony or chitinous case or shield covering the back or part of the back of an animal (as a turtle or crab).

6.8 **Composite-** combining the typical or essential characteristics of individuals making up a group.

6.9 **Crustacean-** any of a large class (Crustacea) of mostly aquatic mandibular arthropods that have a chitinous or calcareous and chitinous exoskeleton, a pair of often much modified appendages on each segment, and two pairs of antennae and that include the lobsters, shrimps, crabs, wood lice, water fleas, and barnacles.

6.10 **Digestate-** product of digesting.

6.11 **Fillet-** to cut, a boneless cut of fish.

6.12 **Head-** the upper or anterior division of the animal body that contains the brain, the chief sense organs, and the mouth.

6.13 **Hepatopancreas-** a glandular structure (as of a crustacean) that combines the digestive functions of the vertebrate liver and pancreas**.**

6.14 **Homogenize-** to reduce the particles of so that they are uniformly small and evenly distributed.

6.15 **Mantle-** a fold or lobe or pair of lobes of the body wall of a mollusk or brachiopod that in shell-bearing forms, lines the shell and bears shell-secreting glands.

6.16 **Pectoral muscle-** any of the muscles which connect the ventral walls of the chest with the bones of the upper arm and shoulder and of which there are two on each side of the human body.

6.17 **Swimmerets-** one of a series of small unspecialized appendages under the abdomen of many crustaceans that are best developed in some decapods (as a

lobster) and usually function in locomotion or reproduction

6.18 **Telson-** the terminal segment of the body of an arthropod or segmented worm.

6.19 **Thorax- 1)** the middle of the three chief divisions of the body of an insect also**,** the corresponding part of a crustacean or an arachnid. **2)** the part of the mammalian body between the neck and the abdomen also**,** its cavity in which the heart and lungs lie.

7.0 INTERFERENCES

7.1 Samples being tested for metals must be processed with a ceramic knife and/or ground with a plastic blade to prevent contamination from metals such as steel or tin.

7.2 Samples being tested for organics must be processed with metal, Teflon, PTFE and or glass utensils. The use of plastics may cause interferences with the analysis of samples.

8.0 SAFETY

8.1 The use of laboratory equipment and chemicals exposes the analyst to several potential hazards. Good laboratory techniques and safety practices shall be followed at all times. Approved PPE, which includes safety glasses, gloves, must be worn at all times in the lab. Lab coats are provided and may be worn. All Personal Protective Equipment (PPE) must be removed before leaving the laboratory area and before entering the employee lounge or eating area. Always wash your hands before leaving the laboratory.

8.2 All standards, reagents and solvents shall be handled under a hood using the proper PPE. All flammable solvents must be kept in the flammable storage cabinet, and returned to the cabinet immediately after use. When transporting chemicals, make sure to use a secure transporting devise and/or secondary outer container.

8.3 The chemist should have received in-house safety training and should know the location of first aid equipment and the emergency spill/clean-up equipment before handling any apparatus or equipment.

8.4 Extreme caution must be taken when using or handling knives, descalers, and grinders to homogenize the biota samples.

8.5 Re-useable cotton mesh glove liners may be worn under latex or PVC gloves as an additional measure when using sharp tools or knives, or when dealing with samples that have sharp teeth, spines, fins, or thorns. The mesh lining can help prevent piercing of the skin in case a tool or sample slips, during dissection or other preparation steps.

8.6 Polychlorinated biphenyls should be treated with extreme caution; as a class of chemical compounds they possess both toxic and suspected carcinogenic

properties.

8.7 All additional company safety practices shall be followed at all times as written in the Pace Analytical Chemical Hygiene Plan.

9.0 EQUIPMENT AND SUPPLIES

9.1 Cutting board-made of either glass or polyethylene.

9.2 Food processor with titanium cutting blade (small), or blender with stainless steel blades (large).

9.2.1 2- Retsch Grindomix (model GM200) with glass and or plastic mixing bowls

9.2.2 1-Kitchen Aid Little Ultra Power

9.2.3 1-Tor Rey (model M22) Large Food Processor

9.3 Knives: ceramic stainless steel, or titanium. (See Section 7.0 for interferences and/or contamination associated with different material knives and blades).

9.3.1 Gerber Stainless Steel Boning knives

9.3.2 Dexter Russel Chopping knives

9.3.3 Oneida Stainless Steel fillet knives

9.3.4 URI Eagle Ceramic Knife

9.4 Necropsy dissection kits

9.5 Analytical balance with precision to 0.01g.

9.6 Labconco multi-hazard glove box.

9.7 Advantage 200 LS Respirator Facepiece

9.8 Bench liner material (Lab Mat) and scissors.

9.9 Aluminum foil.

9.10 Plastic wrap or wax paper.

9.11 Titanium fork.

9.12 Teflon-coated spatula.

9.13 Teflon or stainless steel tweezers and dissection scissors.

9.14 PVC or Latex gloves.

9.15 Ruler.

9.16 Mallet.

9.17 Stainless steel or plastic strainer.

9.18 Salad spinner.

9.19 Pre-cleaned glass sample jars with Teflon or PTFE-lined caps.

9.20 Kim wipes.

9.21 Nylon bristled brushes for cleaning.

10.0 REAGENTS AND STANDARDS

10.1 **Deionized (DI) water**- Deionized (DI) water or reagent water is ASTM Type II laboratory reagent grade water or better (Type I).The Millpore NANO-pure system provides Type I water used in the metals laboratory for rinsing lab glass and plastic ware. Other grades may be used, provided it is first ascertained that the reagent is of sufficiently high purity to permit its use without lessening the accuracy of the determination. If the purity of a reagent is in question, analyze for contamination.

10.2 **Hexane** - Pesticide grade

10.3 **Acetone** - HPLC grade

10.4 **Nitric acid 25%** - Add 250mL concentrated HNO3 to 400mL of reagent water and dilute to 1L in an appropriate flask. (See metals lab for this prepared solution).

10.5 **10% Bleach solution** - Add 100mL of commercial bleach to 500mL of reagent water and dilute to 1 liter in an appropriate beaker or flask**.**

10.6 **Alconox** - cleaning solution.

11.0 SAMPLE COLLECTION, PRESERVATION, SHIPMENT and STORAGE

11.1 Sample collection is not applicable to the Pace laboratory operation.

11.2 Please see the Pace SOP (NE227) that describes the responsibilities of sample custody including all proper documentation, verification, and tracking procedures following Chain of Custody (COC) protocols, sample receipt procedures, and Internal COC procedures for sample tracking include the use of sample tracking logbooks.

11.3 All samples should remain frozen at all times unless being tested. Fish usually arrive whole bodied or already filleted. Once received the sample must be ground and homogenized so that it may be analyzed. The homogenized fish tissue can be held for 6 to 12 months. The fish solvent extracts can be held for 3 months. Some

clients may request that the body and/or head of fish be saved once the fillets are cut out. Other biota material may have other specifications stated specifically for that project.

11.4 If samples are not shipped frozen, they will be stored in freezers at Pace Analytical upon arrival, and until processing. The samples must remain frozen and maintained at < -20˚C. Sample processing and extraction/digestion hold times are suspended by freezing the sample. Hold time monitoring is resumed when samples are removed from freezers for processing and then returned to freezers pending extraction or digestion. The organic hold time is 14 days from sample collection to extraction, and 40 days from extraction to analysis. The metals hold time is six months from sample collection to digestion and analysis. If mercury is to be determined, the hold time is 28 days from sample collection to digestion and analysis.

11.5 Tissue samples: As guidance, a minimum of 50 grams of sample must be collected for organic analyses, and 5 grams for metals analyses, in a glass jar with a Teflon or PTFE lined screw cap. The amount of sample needed, will depend upon the project management plan such as reporting limits and the need for MS/MSD and/or duplicate analyses. Extra sample must be collected, if possible, to allow the laboratory adequate sample volume in case of re-extract and reanalysis is needed. Large whole individual fillets or vegetation may be wrapped in plastic or aluminum foil depending upon the requested analyses. Large crustaceans, reptiles or amphibians may be individually packed in well-labeled Styrofoam coolers.

12.0 QUALITY CONTROL

12.1 Contamination Prevention

12.1.1 If the purity of a reagent is in question, analyze for contamination.

12.1.2 Blades for dissection may need to be re-sharpened between every few samples as needed.

12.1.3 Certain project specific sample preparation (weighing, skinning, compositing and homogenization) is performed in the glove box. Waste from the process must be treated with bleach before disposal. The outside surfaces of the sample containers being processed must be containerized, treated and disinfected before removal from the glove box.

12.2 The procedures described below are general cleaning and pre-processing procedures that are to be followed regardless of the type of tissue being processed. Samples are prioritized by the Laboratory Supervisor or Lab Manager based on hold time and client due date. All weights, measurements and other project required observations are recorded in LIMS.

12.2.1 Wash all utensils, sample processors (blades, blade post, cup and lid) and cutting boards with an Alconox solution and a sponge. Rinse thoroughly with tap water, then with DI water.

12.2.2 If the samples are going to be processed for organic analyses only, rinse

all washed utensils, processor parts and surfaces with hexane followed by rinsing with acetone.

12.2.3 If samples are going to be processed for metal analyses only, rinse all plastic and ceramic utensils with DI water and then Nitric acid 25% solution and then DI water again.

12.2.4 If requested by the client, the equipment or processing blank should be collected at this time by pouring DI water into and out of the processor, over the surfaces of the utensils and over the cutting board. The blank is collected in the appropriate container, at the project specification frequency, for the determinative analysis.

12.2.5 Gloves must be worn when handling tissue samples. Latex gloves may be worn. All gloves must be talc or dust free.

12.2.6 Tissue samples should be partially thawed before starting, to the point where it becomes possible to make an incision in, or cut through, the flesh. When samples are completely thawed they become soft and difficult to cut or fillet. NOTE: If whole bodies are not being processed, and the tissue is partially frozen during dissection, there is less of a chance of puncturing the gut cavity and any internal organs. Inadvertent puncture of the internal organs may contaminate the part(s) of the animal that have been selected for analysis. Also, internal organs may rupture during freezing. If this is observed during dissection, it must be noted in the processing records. Note any morphological abnormalities on the processing records.

12.3 Hold times: The homogenized fish tissue can be held for 6 to 12 months. The fish solvent extracts can be held for 3 months.

13.0 CALIBRATION AND STANDARDIZATION

13.1 Not Applicable

14.0 PROCEDURES

14.1 Fish Tissue Preparation:

14.1.1 Determine the wet weight for each individual fish using a calibrated balance and record in LIMS. The balance should be covered with aluminum foil if aluminum is not a metal of concern. If aluminum is a metal of concern and the sample will not be analyzed for organic compounds the balance should be covered with plastic wrap. If the sample is for both metal and organic compounds, wax paper may be used. Catch any excess fluid coming from the thawing specimen into the wax paper, foil or plastic wrap. All liquid from thawed whole fish must be kept as part of the sample. The technician must remember to zero the balance with the aluminum foil, plastic wrap, or wax paper on it before weighing the specimen. The foil, plastic wrap, or wax paper must be changed after each weighing.

14.1.2 Determine the length of each fish using a ruler, and record in LIMS. Some measurements may, or may not be, a part of the project specifications.

14.1.3 If gender identification is needed this must be done prior to the scaling and filleting processes.

14.1.4 Removal of Scales or Skin: If required by project specifications, the scales and/or skin of the fish will be removed prior to filleting.

14.1.5 Lay the fish on the cleaned, and/or lined, cutting board.

14.1.6 Scrape the fish from tail to head using the electric, automated descaler with ceramic claws to remove the scales. Note: If performing metals analysis, titanium or ceramic must be used.

14.1.7 Rinse the cutting board between fish with DI water and Alconox. If plastic, wax paper, or foil is used, change between fish.

14.1.8 Rinse the outside of the fish with DI water and pat dry with paper towel Place the fish on its side, on a clean cutting board, for filleting or skinning.

14.1.9 To skin the fish, loosen the skin behind the gill cover and pull the skin off toward the tail with a Catfish skinning tool, cutting lightly along the inside of the skin, Slowly separate the skin from the muscle tissue of the body or the fillet.

14.2 Filleting the Fish

14.2.1 Using fresh gloves and the specified knife, make a cut behind the entire length of the gill cover, making sure to cut through the skin, if still attached, flesh, and as close to the bone as possible. Note: If the fish samples are small, and it appears difficult to fillet, or if the amount of the fillet appears to be insufficient for the analysis, consult the Project Manager prior to filleting. In some cases it may be necessary to homogenize the whole body.

14.2.2 Make a cut across the base of the tail fin keeping as close to the caudal fin (tail) as possible. Continue cutting along the underbelly of the fish moving from the head to the tail.

14.2.3 Go back to the cut made at the beginning at the gill cover and slice down the entire length of the fish following along the backbone until reaching the cut previously made across the tail.

14.2.4 Remove the fillet from the fish. Be sure to include the belly flap in each fillet and do not remove the dark muscle tissue in the vicinity of the lateral line from the light muscle tissue that makes up the rest of the muscle tissue mass.

14.2.5 Remove any bones that may be left attached to the fillet. Repeat the fillet

steps for the second side of the specimen.

14.2.6 The general procedure recommended for filleting fish is illustrated in Apendix 1.

14.2.7 Note in the sample processing records in LIMS if the internal organs were ruptured during freezing or if inadvertent puncture of the internal organs occurred during the filleting process, rinse the fillet(s) tissue with DI water.

14.2.8 Cover the balance with the appropriate clean lining, and weigh the fillet(s). Record the fillet(s) weight(s) in the processing records.

14.2.9 If the fillet(s) and/or the carcass are to be homogenized immediately, proceed to Section 14.3. If not, rinse all fish parts with DI water and store in the appropriate container; see Section 9.0 for allowable materials. Note that it may be necessary to chop the fillet(s) or carcass into smaller pieces, with the appropriately cleaned knife, before storage, and before homogenization, so the entire sample will fit into the storage container or the homogenization vessel. If the samples will not be homogenized immediately, the samples must be placed back into the freezer, until homogenization.

14.3 Homogenization

14.3.1 Allow the fillet(s), carcass or whole body to partially thaw. Retain all fluids as part of the sample.

14.3.2 Homogenize whole fish bodies, carcasses, or fish fillets by placing them into the small or large food processor fitted with the appropriate blades. The sample may need to be cut into smaller pieces for processing. Process the sample until it appears to be fully and consistently homogenous. Continue to grind the sample until there are no chunks present in the homogenate. The homogenous nature of the sample is vitally important for reproducible results. Sample should be homogenized fully and thoroughly.

14.3.3 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

14.3.4 Place the individual or composite homogenized samples into the appropriate glass jars to be frozen pending future extraction/digestion. If the samples will not be extracted/digested immediately, the samples must be returned to the freezer until extraction/digestion.

14.3.5 All utensils and equipment must be washed in between samples

according to the procedures described previously in Section 12.2.

14.4 Mollusk (Bivalves) Preparation (Mussels, Clams)

14.4.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 12.2. Obtain samples from freezer.

14.4.2 If required by the project specifications, measure and record the length of the sample shell. Cover the balance with the proper material as described in Section 9.0, and weigh and record the sample weight in LIMS.

14.4.3 Wearing the proper gloves, place the sample on a clean, cutting board. Samples should be partially thawed. If the sample is frozen, it will be difficult to break open the shell. If the sample is excessively thawed, the internal tissue will become soupy and difficult to remove.

14.4.4 If preparing bivalve specimens, use the titanium knife to cut the abductor muscle by sliding the knife through the crevice where the two shells meet. Once the abductor muscle is cut the two shell pieces should come apart easily.

14.4.5 Carefully remove the top shell, and using the Teflon coated spatula, scoop out the internal tissue that is resting on the mantle.

14.4.6 Cover the balance with the proper material and weigh the amount of tissue obtained from the sample. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar.

14.4.7 Since the amount of tissue obtained from one bivalve is generally small, several specimens are frequently combined to make one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be rinsed in between each composite sample.

14.4.8 After the tissue has been removed from all of the specimen shells for one composite or individual sample, place the tissue in the clean small processor with the titanium blade to be homogenized. Grind the sample until it appears to be fully and consistently homogenized and there are no large chunks.

14.4.9 If tissue is being processed for volatile organic carbon (VOC) analysis the homogenization must be done by hand.

14.4.10 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-

homogenized by hand mixing prior to being extracted or digested.

14.4.11 Place the processed samples into the appropriate glass jars to be frozen for future extraction/digestion, and place back into the freezer.

14.4.12 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 12.2.

14.5 Crustaceans (Lobsters, Crabs, Shrimp)

14.5.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 12.2. Obtain samples from the freezer.

14.5.2 If project specifications require gender determination of lobsters, this must be done prior to dissecting. To determine the gender, hold the lobster by the thorax, and flip it over to examine the underneath abdomen, just below the legs and where the abdomen division begins, there is a first pair of swimmerets. The first pair of swimmerets is what is used to distinguish the lobster's gender. If the first pair is soft, has small hairs, and the swimmerets are crossed, it is female. On a male lobster, the first pair of swimmerets is hard and stiff, and generally do not touch.

14.5.3 If the hepatopancreas of the lobster samples is to be analyzed, the lobster samples must be received alive. If the samples are frozen prior to dissection, the hepatopancreas will burst upon thawing making it impossible to remove. To remove the hepatopancreas, the live lobster should be placed on a cleaned cutting board. Wearing the proper gloves, one analyst holds claws out in front of the lobster, while also holding down the lower abdomen and tail. The second analyst takes a titanium-coated knife, and places it on the grove in the outer shell, just behind the head region. Keeping the knife at an angle, the second analyst must push down and forward, to remove the head. Once the head is removed, the hepatopancreas can be seen lying just under the carapace and running the length of the thorax. The hepatopancreas is generally a greenish-yellow color, but there may be some variation. Using the Teflon coated spoon, scoop the hepatopancreas out gently trying not to break it into pieces. Cover the tray of the balance with the proper material, and weigh and record the weight of the hepatopancreas in the processing record, and place it into an appropriate sample jar for freezing and future extraction/digestion.

14.5.4 To remove the edible meat, remove the two claws from the body of the lobster at the joint. Place a piece of lab mat or paper towel over the claw and pound with a mallet. Once the shell is crushed, remove the meat, using the appropriately cleaned tweezers or other tool, making sure to get all the meat in the joints and arms. Cover the balance tray with the appropriate material and record the total tissue weight arms. Record this weight with the previously recorded information from the two claws and sample processing record.

14.5.5 Remove the abdomen and telson from the rest of the outer shell by pulling the lobster apart. Using the titanium coated knife, cut through the

center underside tissue of the lobster and laterally along the exoskeleton of the tail. Once the abdomen and tail have been cut open, separate the shell from the edible meat using cleaned utensils. Any eggs found in the female lobsters will have to be removed and discarded or sampled separately. Cover the balance tray with the appropriate material, and record the weight of the tissue obtained from the abdomen and telson on the processing record. The sample may now be stored pending homogenization in the appropriate jar.

14.5.6 If removing tissue from crabs, break off all legs and claws. Squeeze, pull, or pick all the tissue out of the legs and claws. Pull apart the outer shell. Scoop out the tissue using a Teflon coated spatula. Cover the balance tray with the appropriate material, and record the weight of the tissue obtained from the abdomen and telson on the processing record. The sample may now be stored pending homogenization in the appropriate jar.

14.6 Mammals (Mice, Mink, Muskrat, Shrew)

14.6.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 12.2. Obtain samples from the freezer.

14.6.2 Place the first specimen partially thawed to be processed, and all equipment needed into the glove box/Bio-hood on a freshly laid out lab mat (Blue diaper).

14.6.3 Once all materials are in the glove box and set up for use, seal the transfer box and ensure the motor blower is on. Over tightening of the outer or inner door knobs is not necessary to achieve a good seal. Place your hands into the gloves attached to the glove ports and place Latex gloves over the glove port gloves for use. The outer Latex gloves will need to be changed in between each sample.

14.6.4 If the gender of the mouse or shrew needs to be determined, turn the animal over and note the length of the anus and the distance of the anus from the tail. If the anus is elongated in shape and does not touch the base of the tail, testicles and a large genital papilla are visible, and there are no nipples, the animal is male. If the anus is round in shape and almost touches the base of the tail and/or there are nipples (up to five sets), the animal is female. If the animal is very small, young or immature and a gender determination cannot be made, note that the gender is non determinable. Record the gender observations on the processing records.

14.7 Organ Dissection/Processing

14.7.1 If the mammal is being dissected for Brain, Liver, Kidney, Heart, Lung, or Adipose (Fat) tissue, each organ will need to be harvested.

14.7.2 Place the animal on its back with forceps. Pinch the skin at the base of anus and carefully make an incision at the tail end, and cut just below the skin along the abdomen and past the chest cavity. Cutting the skin flap

at the abdomen cavity carefully separate the adipose tissue from the muscle tissue. Below it should be a white/yellow material. Take this material out.

14.7.3 Identify each organ and remove them from the abdomen cavity.

14.7.4 Weigh and record the weight of the mammal organs and place into the appropriate container.

14.7.5 The rib cage will need to be cut with scissors. Once chest cavity is open, remove the heart and lungs.

14.7.6 Weigh and record the weight of the mammal organs and place into the appropriate container.

14.7.7 Since the amount of tissue obtained from one animal may be small, manually grinding of the organs may need to be done at the time of extraction.

14.7.8 Place the processed samples into the appropriate glass jars to be frozen for future extraction/digestion into the freezer.

14.7.9 Before removing any equipment all utensils and equipment must be washed with DI water and 10% bleach solution.

14.7.10 All disposable materials must be double bagged for disposal.

14.8 Whole Animal Processing:

14.8.1 If skinning of the mammal is required, carefully make an incision at the tail end and cut just below the skin along the back, from one hind leg to the other. Make another cut from one hind leg to one front leg and repeat the cut on the other side of the animal. Starting from the tail, lift the skin flap, and carefully separate the skin from the muscle tissue below. Pull the skin forward from the tail to the head to expose the back tissue of the animal. Repeat the procedure on the stomach side of the animal. Note: it may be very difficult to remove the skin from the legs, head, and tail. If some skin cannot be removed, note this on the processing records.

14.8.2 Weigh and record the weight of the mammal on the processing records. Depending upon the size of the mammal, it may need to be chopped into small pieces before being ground. Generally, mice and shrew can be quartered before homogenization if needed.

14.8.3 Put the whole body or chopped sample into the cup of the grinding unit. Turn the grinding unit on low speed and gradually increase the speed to homogenize the sample being careful to minimize any splatter or outside contamination. Homogenize until a uniform consistency is achieved.

14.8.4 Transfer the homogenized sample from the cup to the pre-labeled sample jar using the appropriate utensil. Clean the outside of the sample

jar with the 10% bleach soaked Kim wipe.

14.8.5 To clean the grinding unit in between samples, remove as much residual tissue on the blade as possible by operating the unit at low or medium speed, using DI water and 10% bleach. Rinse unit with DI water if metals are being done and/or hexane or acetone for organics.

14.8.6 Repeat steps 14.9.2 through 14.9.5 until the samples are complete.

14.8.7 Since the amount of tissue obtained from one mouse or shrew may be small, several specimens may be combined to make one sample, as required by project specifications. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be cleaned in between each composite sample.

14.8.8 If several specimens will be composited to make one sample, follow the applicable Sections of 14.9.2 through 14.9.5, for each of the specimens. The tissue obtained from each specimen may be weighed and recorded individually, then totaled for the composite weight. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight in LIMS.

14.8.9 Place the processed samples into the appropriate glass jars to be frozen for future extraction/digestion, placed back into the freezer.

14.8.10 Before removing any equipment all utensils and equipment must be washed with DI water and 10% bleach.

14.8.11 All disposable materials must be double bagged for disposal.

14.9 Reptiles and Amphibians (Frogs and Turtles)

14.9.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 12.2. Obtain samples from the freezer

14.9.2 Wearing the proper gloves, place the turtle sample on the cleaned cutting board. The turtle should be partially thawed. If the turtle is frozen, it will be difficult to remove the muscle. If the sample is excessively thawed, the internal tissue will become soupy and difficult to remove.

14.9.3 Take all project required measurements. The distance between the anterior and posterior edge of a turtle carapace (top of shell) should be measured with a ruler and recorded on the processing records. If the entire mass of the turtle, including the shell, needs to be recorded, cover the balance with the proper material and weigh and record this weight in LIMS.

14.9.4 Since the bottom of shell and carapace are extremely dense and difficult to cut through with normal dissecting tools, the muscle tissue of the turtle must be removed by cutting the body of the turtle away from the shell. Insert a knife, made of the proper material, into the skin of the turtle, close to the shell on the lower half of the body. Slowly, cut along the

entire circumference of the shell. Repeat the procedure on the upper half of the body, on both sides of the shell.

14.9.5 With dissection scissors or a ceramic or titanium paring knife of the proper material, remove the skin from the hind limbs, tail, and fore limbs and neck. Remove any visible muscle tissue within the carapace. Most of this tissue will be found in the upper portion of the carapace around the pectoral area.

14.9.6 Using the appropriate utensils, remove the muscle tissue from the tail, neck, hind limbs, and fore limbs, including the feet, leaving bone and claws behind.

14.9.7 Cover the balance with the proper material and weigh the amount of tissue of the turtle sample. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar.

14.9.8 If processing frogs, allow the frogs to partially thaw, take the project specific measurements, and record them in LIMS. The number of frogs required to make up one sample, and the weight and length of the individual frogs, must be taken and recorded, if specified. In all cases, the skin must be removed from the frog prior to processing and chopped into smaller pieces, due to its thickness. It will then be added to the processor with the whole body of the frog, or it may be discarded depending upon the project specifications.

14.9.9 To skin the frog, make an incision, using the proper utensils, and cut into an area where there is an excess of skin, most likely around the neck. Slowly, pull the skin off of the frog using dissecting scissors, or a ceramic or titanium paring knife, as needed. Once skin is removed, chop it up into tiny pieces using the appropriate knife and set it aside to be processed with the whole frog body.

14.9.10 Cover the balance with the proper material and weigh the amount of tissue obtained from the frog samples if the tissue and the whole body will not be processed. Record the weight along with the information previously recorded on the processing records. The sample may now be stored pending homogenization in the appropriate jar.

14.9.11 Since the amount of tissue obtained from one small turtle or frog may be insignificant, several specimens may be combined to make up one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be rinsed in between each composite sample.

14.9.12 If several specimens will be composited to make up one sample, the tissue obtained from each specimen may be weighed and recorded individually, then totaled for the composite weight. If only the composite weight is sufficient for the project specifications, weigh the entire composite and record that weight in LIMS.

14.9.13 After the tissue has been removed from all of the specimens, homogenize the muscle tissue, and skin if required, by placing it into the small or large food processor fitted with the appropriate blades. The sample may need to be cut into smaller pieces for processing. Grind the sample until it appears to be fully and consistently homogenous. Continue to grind the sample until there are no chunks present in the homogenate.

14.9.14 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

14.9.15 Place the processed samples into the freezer to be frozen for future extraction/digestion.

14.9.16 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 12.2.

14.10 Macro Invertebrates (Benthic Worms, Eels, Insects and other Biota)

14.10.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 12.2. Obtain samples from the freezer.

14.10.2 Cover the balance tray with the appropriate material and record the weight of the invertebrate sample. Since the weight obtained from one invertebrate (benthic worm, insect, biota) may be small, several invertebrates may be combined to make one sample. In many cases, several invertebrates of the same species and sample location are delivered to the laboratory in one sample jar. Each specimen from this jar must be weighed, if requested, and composited to form one homogenized and unique sample. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight. Utensils do not need to be rinsed between the individual samples or specimens that comprise one composite, but utensils must always be rinsed between each composite sample.

14.10.3 Invertebrates such as eels must be chopped into smaller pieces before homogenization. This is generally due to the length of the specimen and the thickness of the skin.

14.10.4 Place the weighed specimen into the clean small processor with the titanium blade to be homogenized. Process the sample until it appears to be fully and consistently homogenized and there are no large chunks.

14.10.5 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite homogenates must be prepared from equal weights of individual

homogenates. All individual weights that make up one composite must be recorded, if required, or one composite weight may be recorded. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

14.10.6 Place the processed samples into the appropriate glass jars to be frozen for future extraction/digestion, into the freezer.

14.10.7 All utensils and equipment must be washed in between samples according to the procedures described previously in Section 12.2.

14.11 Vegetation (Coastal and Wetland Grasses/Plants)

14.11.1 Wash all utensils, the cutting board, and surfaces as previously described in Section 12.2. Obtain samples from the freezer.

14.11.2 Wearing the appropriate gloves, plants must be rinsed with DI water to remove soil, silt, small insects, and other debris. Place the plants in a stainless steel or plastic strainer, depending on the determinative sample analysis, and rinse thoroughly with DI water. If analyzing the sample for both metals and organic compounds, rinse the plants carefully over a sink, being sure not to touch the sides of the sink with the plant sample.

14.11.3 Depending on the size and texture of the plants, some may be homogenized in the small food processor with the titanium blade. Samples such as long grass will have to be chopped into small pieces (approximately ½ inch) using titanium or ceramic knives. Leaves can generally be homogenized in the small food processor without pre-cutting.

14.11.4 Cover the balance tray with the appropriate material and record the weight of the plant sample. Since the weight obtained from one plant may be small, several plants may be combined to make one sample. Utensils do not need to be rinsed between the individual samples that comprise one composite, but utensils must always be rinsed in between each composite sample.

14.11.5 If several plants will be composited to make one sample, the weight of each specimen may be recorded individually, and then totaled for the composite weight. If only one composite weight is sufficient for the project specifications, weigh the entire composite and record that weight in LIMS.

14.11.6 After the plant weight for one composite or individual sample has been recorded, place the plant(s) in the clean small processor with the titanium blade to be homogenized, or place them onto the cleaned cutting board to be chopped. Grind or chop the plants until they appear to be fully homogenized.

14.11.7 Individual homogenates may be processed further to prepare composite homogenates as required by project specifications. Composite

homogenates must be prepared from equal weights of individual homogenates. If required, all individual weights that make up one composite must be recorded, otherwise one weight may be recorded for the composite. If individual or composite homogenates were frozen prior to extraction/digestion, these homogenates must be thawed and re-homogenized by hand mixing prior to being extracted or digested.

14.11.8 Place the homogenized plants back into the freezer to be frozen for future extraction/digestion.

14.11.9 All utensils and equipment must be washed between samples according to the procedures described previously in section 12.2.

15.0 CALCULATIONS

15.1 Not Applicable

16.0 METHOD PERFORMANCE

16.1 Not Applicable

17.0 POLLUTION PREVENTION

17.1 Refer to SOP Pace054 and Pace089 for instructions on the disposal of waste generated during the procedures previously mentioned.

18.0 DATA ASSESSMENT AND ACCEPTANCE CRITERIA FOR QUALITY CONTROL MEASURES

18.1 Not Applicable

19.0 CORRECTIVE ACTIONS FOR OUT OF CONTROL DATA

19.1 Not Applicable

20.0 CONTINGINCIES FOR HANDLING OUT-OF-CONTROL OR UNACCEPTABLE DATA

20.1 Not Applicable

21.0 WASTE MANAGEMENT

21.1 Refer to SOP Pace054 and Pace089 for instructions on the disposal of waste generated during the procedures previously mentioned.

22.0 REFERENCES

22.1 NELAP “Quality Systems” Manual, 2005.

22.2 U.S.EPA SW-846 "Test Methods for Evaluating Solid Waste; Volume 1B Laboratory Manual Physical/Chemical Methods", Office of Solid Waste and Emergency Response, Third Edition, Final Update III, December 1996.

22.3 EPA/6OOlR-961027, Guidance for the Preparation of Standard Operating Procedures (SOPS) for Quality Related Documents, 1996.

22.4 US EPA 823-R-95-007, "Guidance for Assessing Chemical Contaminated Data for Use in Fish Advisories", Volume 1: Fish Sampling and Analysis 2nd Edition, Office of Science and Technology, Office of Water, 1995.

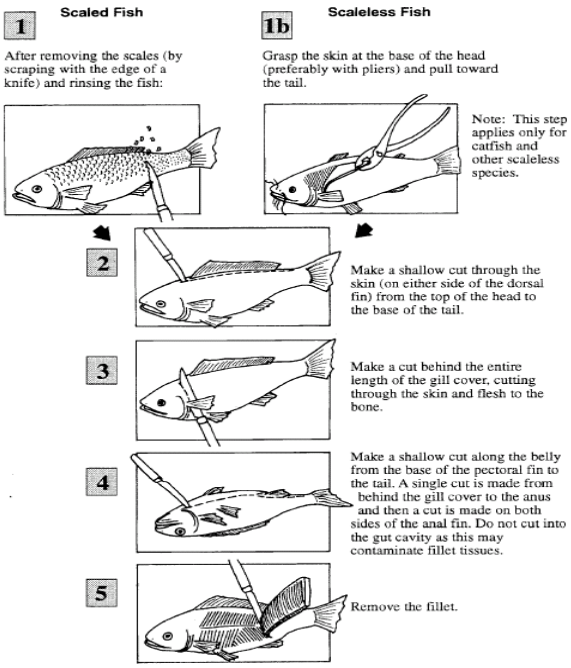
22.5 U.S. EPA, 1991d

23.0 ATTACHMENTS

23.1 Fish Filleting Diagram

23.2 Fish External & Internal Anatomy

Fish Filleting Diagram



23.2 FISH EXTERNAL/INTERNAL ANATOMY

EXTERNAL ANATOMY

1. Remove one fish from the storage tank, place in dissecting pan. Make sure fish is euthanized prior to any dissection.

2. Locate all fins (Figures 1a and 1b):
   Paired: pectoral (caudal to head, located ventrolaterally)
   pelvic (cranial to anus, located ventrolaterally)

   Single: dorsal (caudal to head on dorsal midline)
   adipose (caudal to dorsal fin on dorsal midline; salmonids)

   Anal: (Caudal to anus on ventral midline)

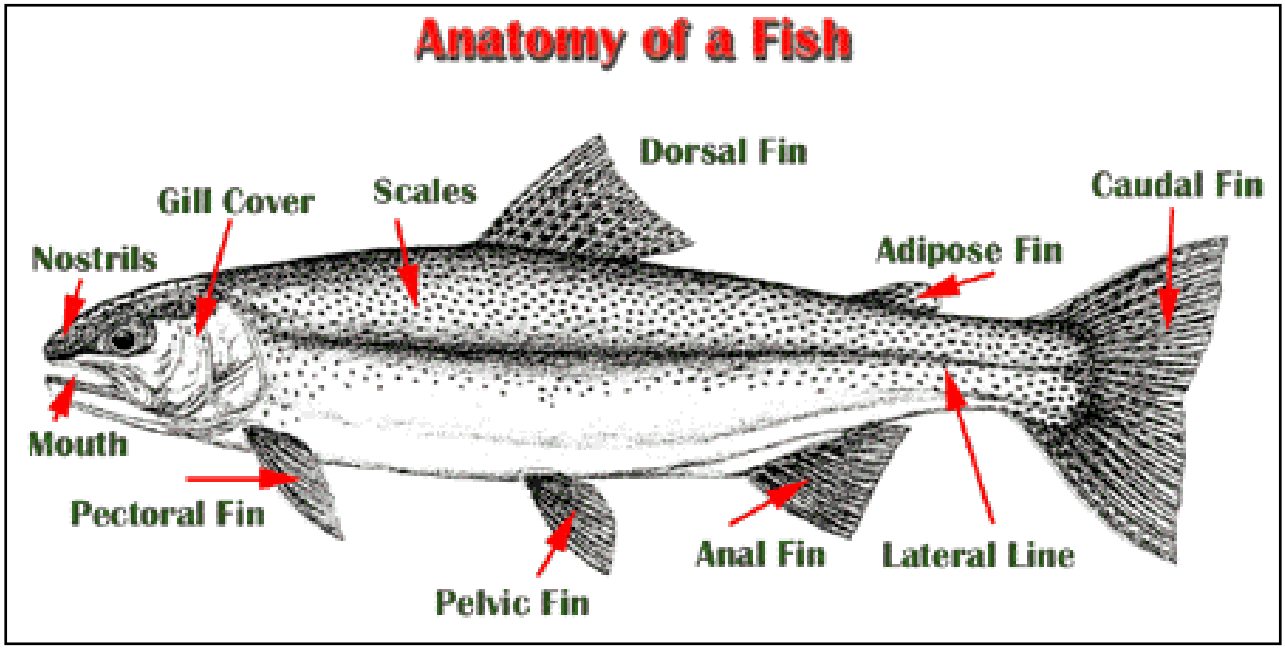


Figure 1a. Anatomy of a Fish (typical salmonid)

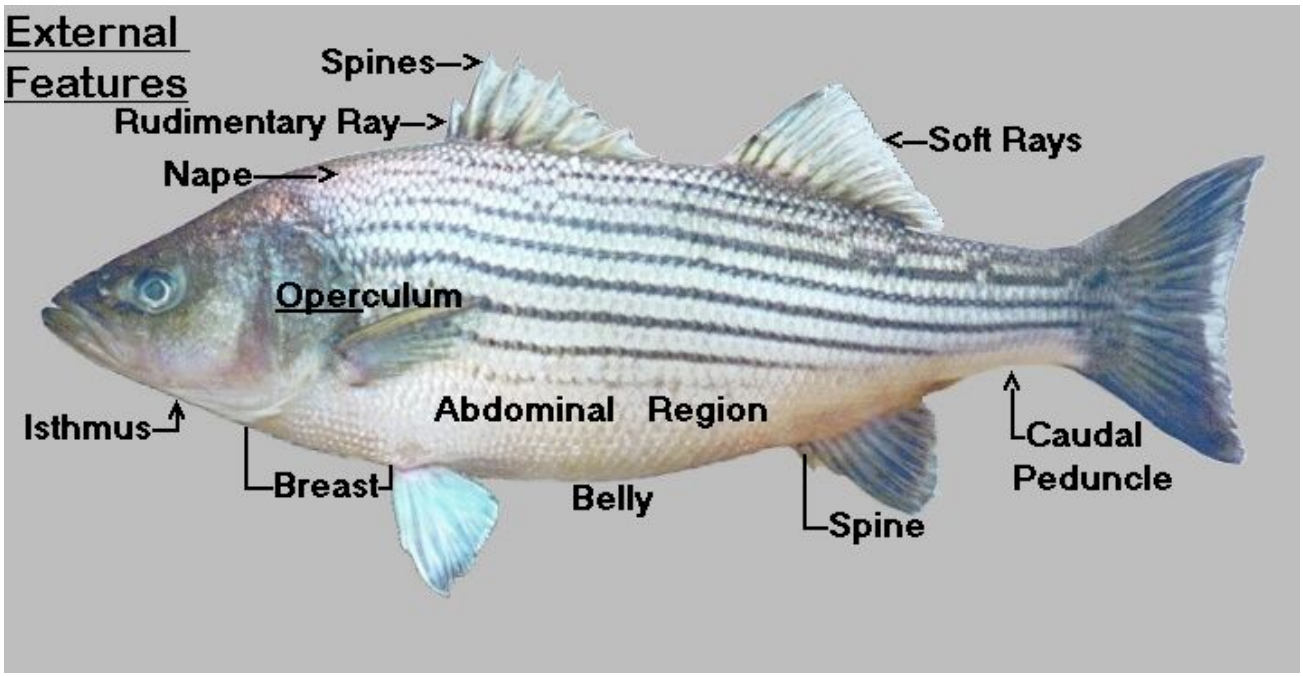


Figure 1b. External Anatomy of Striped Bass

3. Find the lateral line located laterally at mid-body running from head to tail. It arches dorsally over the operculum.

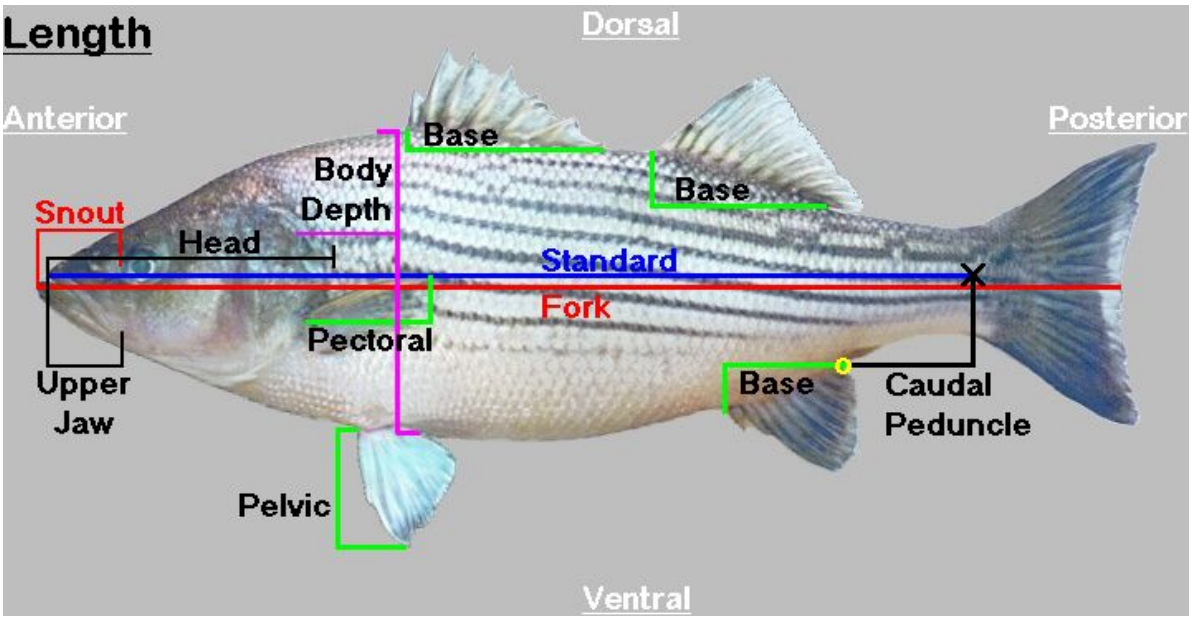


Figure 1c. Typical Measurements Locations

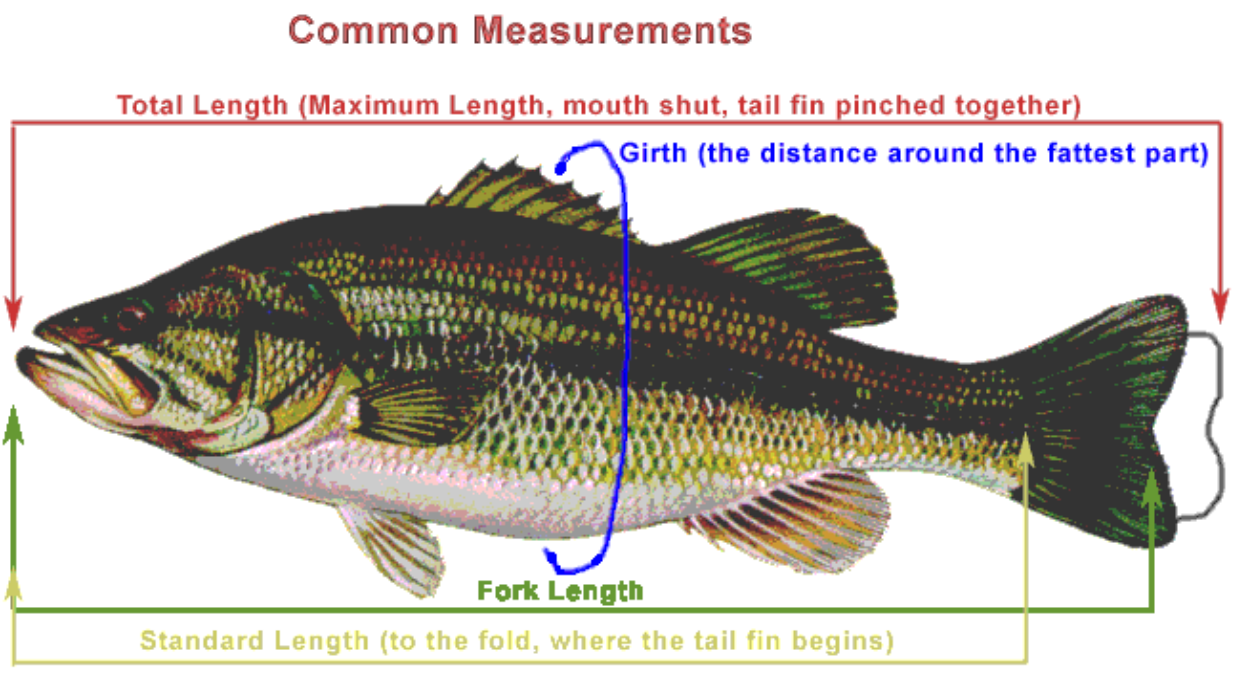
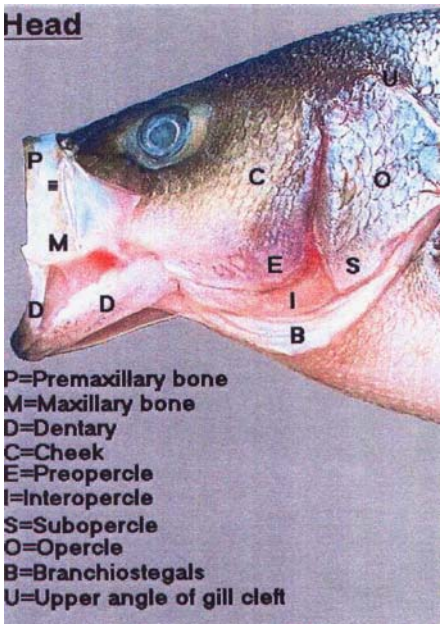
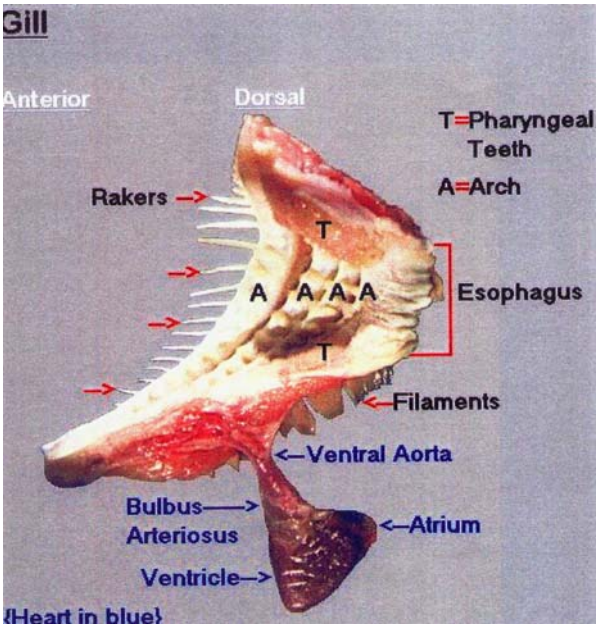


Figure 1d. Typical Measurements of Large Mouth Bass

4. The operculum covers the gills. Lift the opercular flap and identify the bony gill arches, cartilagenous gill filaments, and primary lamellae projecting off the gill filaments (Figures 1f, 1g)





5. Lay the fish on its right side with the head to your left. Open the body cavity with three cuts (Figure 1h). The first cut should originate just craniad to the anus and run cranial to a point ventral to the operculum. The second cut originates from the same point as the first and runs craniad along the dorsum of the body cavity to a point just dorsal to the operculum. The third cut connects the first two. All cuts should be made carefully with the blunt tip of the scissors in the body cavity while applying slight upward pressure to avoid damaging internal organs. Lift off the body wall.

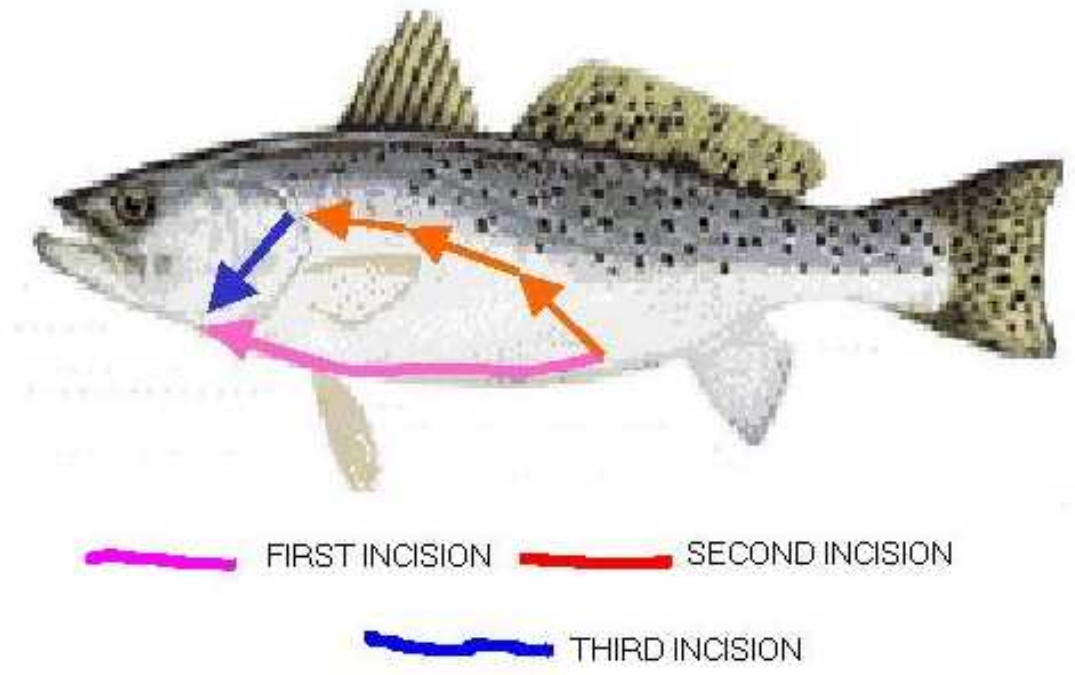
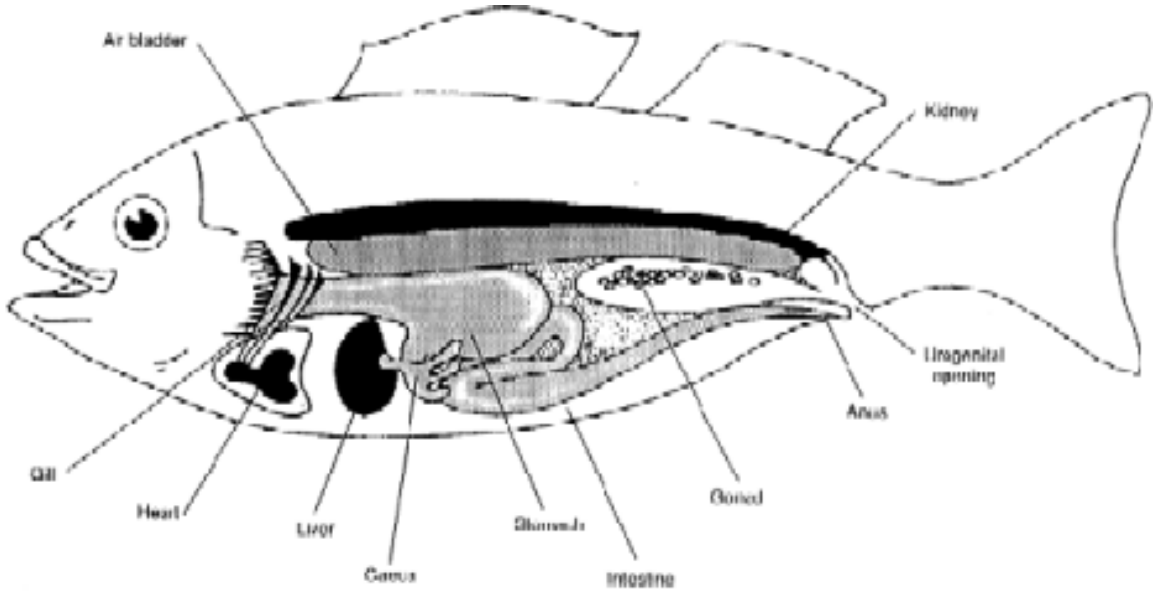
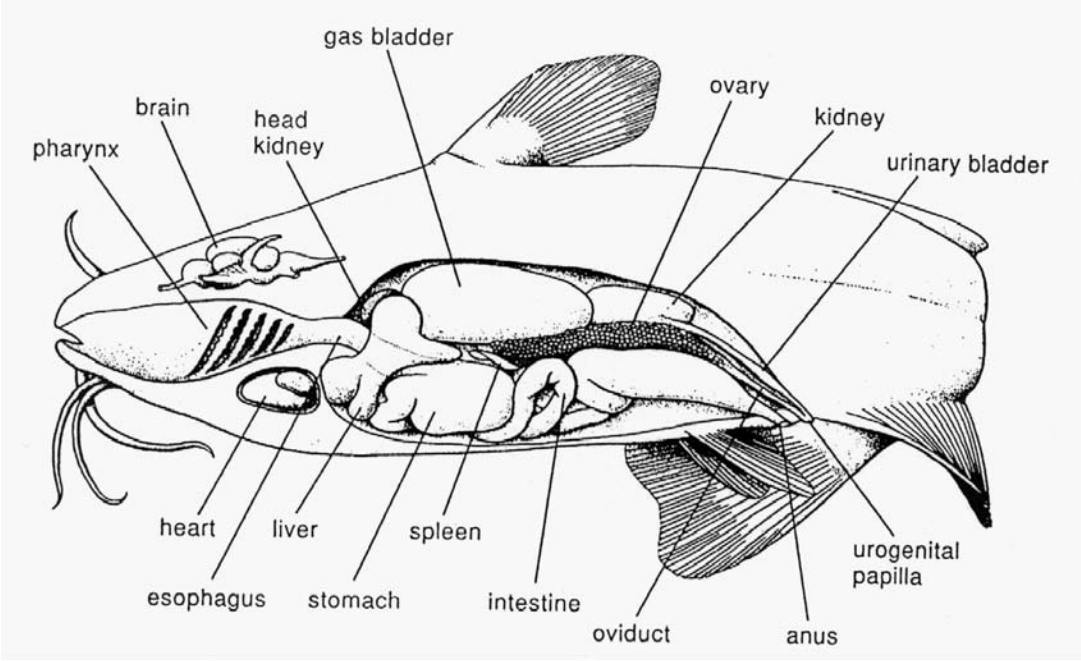


Figure 1h. Incisions to Expose Abdominal Cavity

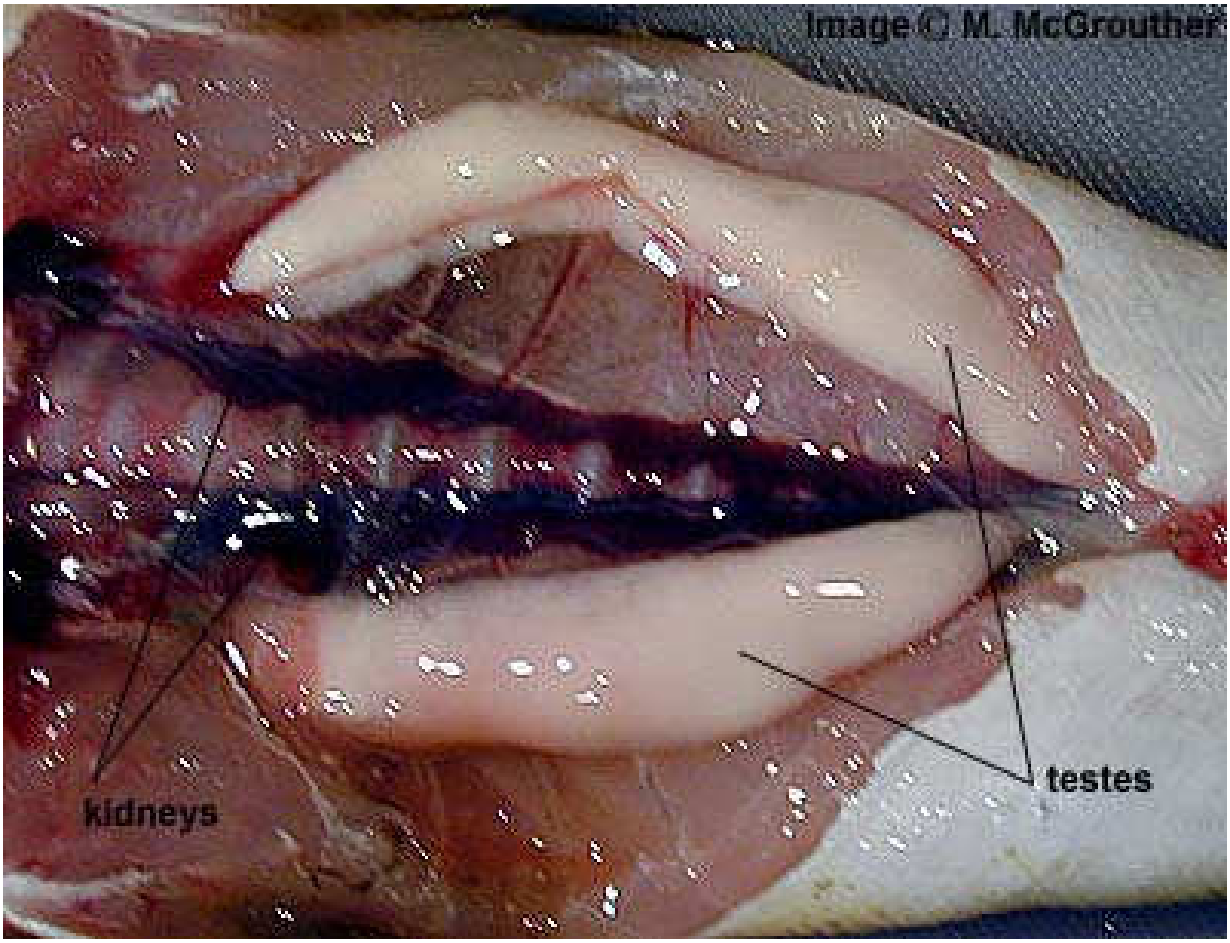
INTERNAL ANATOMY



Identify the gastrointestinal tract (Figures 1i-1,2). Pass a blunt probe through the oral cavity, pharynx, esophagus and into the stomach. Many fish species have pyloric cecae, which are blind sacs projecting from the aborad portion of the stomach. The stomach empties into the intestine, a long tubular structure supported by thin membranes called mesenteries. The intestine terminates at the anus. In fish the intestine is not divided into three distinct regions. The length and complexity of the intestine is directly proportional to the amount of plant matter consumed (herbivorous species have longer intestines). Open the stomach and intestines and note the normal texture and appearance of the lining, or mucosa. The intestinal mucosa will often exhibit lesions when enteric of systemic disease is present. The spleen is a small dark red organ attached to the mesenteries just caudal to the stomach. There may be more than one spleen. The main auxiliary digestive organs are the liver and pancreas. The liver is a large, tan, often leaf-shaped organ just caudal to the heart. The liver is a good site to see many lesions and is also a good site from which to isolate bacterial and viral pathogens. The location and size of the pancreas varies by species. The most common location is interspersed within the liver parenchyma. It may or may not be grossly visible. Cut the intestine near the anus, cut the esophagus and remove the gastrointestinal tract.



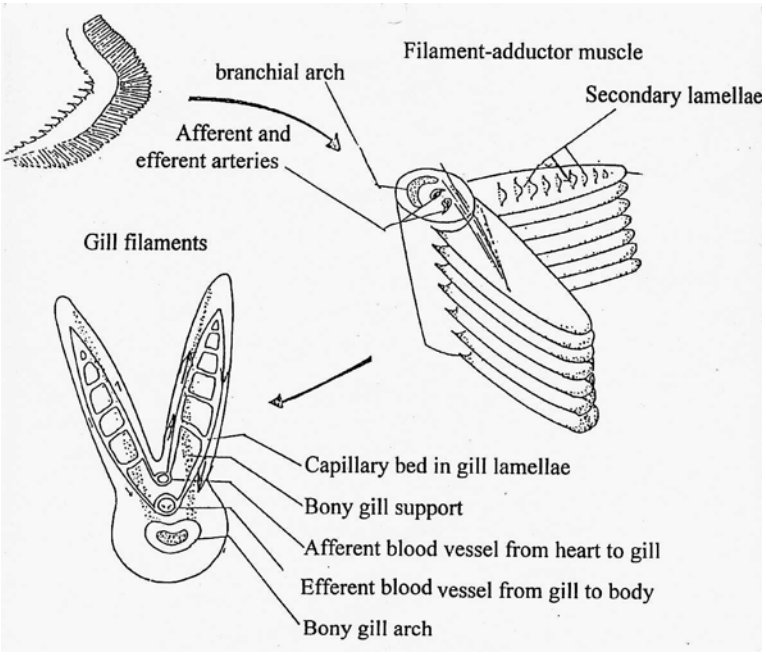
7. Locate the gonads, either ovaries or testes. Ovaries will appear as numerous spherical structures that may comprise up to 70% of body weight. Testes may comprise up to12% of body weight. In mature animals they will appear as a soft white organ suspended from the dorsal body wall. Also, if you don't see either of these organs, you might be working with an immature specimen. Note body length and compare to literature on the species/specimen you are working with.



The gonads and kidneys of an Eastern Blue-spotted Flathead. The gonads (testes) are the large, pale organs and the kidneys are the red tissue either side of the backbone.

8. Along the dorsum of the body cavity lies the swim bladder. It is a thick-walled white organ. Occasionally you may see hemorrhages in the swim bladder.

9. The kidneys also lie in the dorsum of the body cavity. The head kidney and trunk kidney are roughly divided by the swim bladder. In some species (e.g., salmonids) the kidneys are almost fused. The kidneys often exhibit lesions, and the trunk kidney is usually the preferred site for obtaining bacterial and viral cultures. In most fish we work with in this lab, the head kidney and trunk kidney appear fused.



10. The heart lies just caudal to the gills (return to previous figure, Figure 1i). The heart is enclosed in a thin-walled sac, the pericardium. Open the pericardium and examine the heart in situ. Blood returns from the body wall to the sinus venosus, a thin-walled chamber which empties into the atrium. The sinus venosus might be difficult to identify. The atrium pumps blood to the ventricle. The atrium lies cranial and dorsal to the ventricle. The ventricle is the main pump and largest part of the heart. Blood flows from the ventricle craniad to the bulbus arteriosus. The thick-walled elastic bulbus helps regulate blood pressure as blood leaves the heart. As the bulbus passes through the pericardium en route to the gills it becomes the ventral aorta.